Research article

EFFECT OF *Trichoderma* ISOLATES ON *Sclerotium rolfsii* Sacc.

P. Adhikari*, S. M. Shrestha, H. K. Manandhar, and S. Marahatta
Agriculture and Forestry University, Rampur, Chitwan, Nepal

*Corresponding author: adhikari_pk@yahoo.com
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ABSTRACT

Sclerotium rolfsii Sacc. is one of the most important plant pathogens commonly causing root rot, stem rot, wilt and foot rot in various crop species. A wide range of host, profuse mycelial growth, and ability to produce persistent sclerotia makes them difficult to control. Biological control using *Trichoderma* could provide a sustainable tool for the management of diseases caused by *S. rolfsii*. One hundred four *Trichoderma* isolates were evaluated against *S. rolfsii in vitro* by dual culture. Of them, 30 *Trichoderma* isolates found effective were selected to study parasitization of sclerotia of *S. rolfsii in vitro*. Similarly, four *Trichoderma* isolates found effective *in vitro* were used to study their ability to parasitize sclerotia of the pathogen in soil. *Trichoderma* isolates varied significantly ($p<0.001$) for both the inhibition percentage and biocontrol index. However, they showed a differential effect as *Trichoderma* isolate T_{73}, Forest soil had highest inhibition (96.96%), while *Trichoderma* isolate T_{49}, Bhaktapur showed the highest bio-control index (99) against *S. rolfsii*. *Trichoderma* isolates T_{49}, Bhaktapur and T_{87}, Forest soil showed promising results in controlling *S. rolfsii in vitro*. Both isolates were also able to parasitize and reduce the germination of sclerotia in the soil. The present study demonstrated that selected *Trichoderma* had potential in controlling *Sclerotium rolfsii*.

Keywords: Parasitization, *Sclerotium rolfsii*, sclerotia, *Trichoderma*

INTRODUCTION

The genus *Trichoderma* consists of anamorphic fungi isolated primarily from soil and decomposing organic matter, with teleomorphs, belonging to the ascomycete genus *Hypocrea*. *Trichoderma* species are being used alone or in combination with compatible chemicals for the control of several soil borne diseases such as collar rot, root rot, wilt etc. *Trichoderma* strains exert bio-control against fungal plant pathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and inducing systemic resistance and antibiosis, or directly, by mechanisms such as mycoparasitism (Benitez et al., 2005). These indirect and direct mechanisms may act coordinately and their importance in the bio-control process depends on the *Trichoderma* strain, antagonized fungus, crop plant, and the environmental conditions, including nutrient availability, pH, temperature, and iron concentration (Harman, 2000; Harman et al., 2004). Production of specific compounds and metabolites, such as plant growth promoting hormones, hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen permeases are due to activation of each mechanism (Harman et al., 2004). There also are reports of enhanced plant tolerance to abiotic stresses during plant growth because of improved root growth or improvement in water holding capacity of plants or enhancement in potassium uptake (Bae et al., 2009; Yildirim et al., 2006).

The study on *Trichoderma* species for potential biocontrol agent is getting priority due their great antagonistic abilities against soilborne plant pathogens, including *S. rolfsii.* The pathogen commonly occurs in the tropics and other warm regions of the world causing root rot, stem rot, wilt and foot rot in agricultural crops (Farr et al., 1989). A wide range of host, profuse mycelial growth, ability to produce persistent sclerotia (Asghari and Mayee, 1991; Kokub et al., 2007) and typical damaging symptoms of yellowing and wilting contribute to the greater economic loss. Fungal bio-control agents may directly or indirectly kill sclerotia or mycelium of *S. rolfsii* (Prasad & Naik, 2008). According to Prasad & Naik (2008), *S. rolfsii* produced lectins that acted as recognition factors for fungal bio-control agents. Among the fungal bio-control agents, *Trichoderma* species are the most widely studied and used. In direct confrontation with fungal pathogens *Trichoderma* employs mycoparasitism and antibiosis (Vinale et al., 2008). In the process of mycoparasitism, *Trichoderma* produces various kinds of cell wall degrading enzymes such as chitinase, cellulose, glucanase etc. Upon secretion of these enzymes by *Trichoderma*, cell wall of the pathogen rupture (Kubicek et al., 2001). In a direct interaction with sclerotia of *S. rolfsii*, hyphae of *Trichoderma* penetrate the rind and the

cortex and lyse the medullary tissue (Prasad and Naik, 2008). The present study was conducted to identify potential *Trichoderma* isolates against *S. rolfsii*.

MATERIALS AND METHODS

Isolation of *Trichoderma*

Trichoderma selective medium (TSM) was used for isolation of *Trichoderma*. The ingredients used in the medium are $MgSO_4.7H_2O$ (0.2 g), K_2HPO_4 (0.9 g), KCl (0.15 g), NH_4NO_3 (1.0 g), glucose (3.0 g), streptomycin sulphate (0.02 g), captan (0.2 g), rose bengal (0.15 g), agar-agar (20 g) and distilled water (1 litre). The recipe was dissolved in 1000 ml distilled water and autoclaved at 121°C, 15 PSI for 30 minutes. Then streptomycin sulphate, captan and rose bengal was added prior to pouring into Petri plates. Approximately 20 ml medium was poured into the Petri plates and left overnight to ensure contamination free plates. One gram of soil was serially diluted upto 10^{-3} dilution. Twenty microliters soil suspensions of 10^{-3} dilution were taken with the help of micro pipette and placed onto TSM plates and spread with the help of glass rod until the suspension dried-up. The entire process was carried out under laminar flow. The plates were incubated at 25 ºC and examined daily for the growth of *Trichoderma* for 5 days. Each *Trichoderma* colony that appeared in the TSM plate was isolated and cultured in PDA slant. About 67 *Trichoderma* isolates were isolated from soil samples. Similarly, 20 *Trichoderma* isolates were obtained from National Plant Pathology Research Centre of NARC, Khumaltar and 17 isolates from Plant Pathology Department, AFU. A total of 104 *Trichoderma* isolates were used in the study.

Isolation of *Sclerotium rolfsii*

S. rolfsii was isolated from infected lentil roots collected from a field at Rampur, Chitwan. Plant roots were washed and cut into small sections. The cut root samples were surface sterilized by dipping in 1 % sodium hypochlorite for 1 min, rinsed with sterile distilled water, and plated on Petri plates with moist two layers of blotter papers. The plates were incubated at 25±1 ºC in an incubator until sclerotia were produced and the sclerotia produced were transferred to potato dextrose agar (PDA) to obtain pure culture. The pure culture was maintained at 4 ºC.

Dual culture of *Trichoderma* isolates with *Sclerotium rolfsii*

One hundred four *Trichoderma* isolates were tested *in vitro* by dual culture for interactions with *S. rolfsii* isolated from lentil root. Both *Trichoderma* isolates and *S. rolfsii* were cultured and maintained on PDA slants. The experiment was carried out with three replications in a completely randomized design. The PDA disc of five mm diameter, cut from the edge of actively growing colony (3-day-old) of each *Trichoderma* isolate and the pathogen *S. rolfsii*, was placed at the opposite direction in 9 cm Petri plates (slightly modified from Szekeres et al., 2006). Mycelial discs of *Trichoderma* isolates were placed 2 cm apart from the Petri plate periphery and 5 cm apart from the pathogen. The antagonistic process was observed and followed by taking photographs of the plates with a camera in a fixed stand using the same distance (18 cm). On the digital images, the colonies of the *Trichoderma* strains as well as the total areas occupied by the colonies of both *Trichoderma* and *Sclerotium rolfsii* were drawn around and measured by ImageJ software with the use of a freehand selection tool. The *in vitro* antagonistic abilities of the examined *Trichoderma* isolates were characterized by the biocontrol index (BCI) values calculated according to the following equation:

$$BCI = \frac{A}{T} \times 100$$

Where, A = Area of the *Trichoderma* colony, T = Total area occupied by the colonies of both *Trichoderma* and the pathogen

Percent inhibition of mycelial growth of pathogen was also calculated by using following formula:

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = Percent inhibition in mycelial growth, C = Growth of pathogen in control plate, T = Growth of pathogen in dual culture plate

In vitro evaluation of selected *Trichoderma* isolates for sclerotial parasitization of *Sclerotium rolfsii*

Based on the screening of 104 isolates of *Trichoderma* in dual culture, thirty isolates were selected for their ability to parasitize sclerotia of *S. rolfsii* under *in vitro* conditions. The pathogen was grown in PDA and matured sclerotia were harvested one week after incubation. Twenty-five sclerotia of *S. rolfsii* of uniform size were placed on six-day-old cultures of *Trichoderma* isolates grown on PDA plates and incubated at 25 °C for 7 days. The viability of *S. rolfsii* sclerotia was evaluated by examining them under a stereomicroscope for their germination and hyphal growth. Sclerotia was considered viable when hyphae grew out of them.

Evaluation of selected *Trichoderma* isolates for sclerotial parasitization in pot

Based on the *in vitro* results of sclerotial parasitization, four *Trichoderma* isolates viz. T_{49}, Bhaktapur; T_{31}, Banke; T_{40}, Jumla; and T_{87}, Forest soil were selected for *in vivo* evaluation in pot culture. Mass culture of both the antagonists and the pathogen was prepared in the mixture of rice bran and rice husk in 1:5 proportions. Twenty-five gram of the mixture was autoclaved (121 °C at 15 psi for 60 minutes) twice on two consecutive days and allowed to cool under sterile conditions. Then, the mixture was inoculated with 5 mm diameter-sized agar plugs of 3-day-old cultures of both *Trichoderma* and *S. rolfsii* in separate polybags and incubated for 10 days at 25 °C in an incubator.

Pots (12 cm diameter) were filled with sterilized soil and inoculated with the culture of *S. rolfsii* at the rate of 2 g per kg soil. Ten lentil seeds were sown per pot. Seedlings were allowed to grow until they died due to infection by *S. rolfsii*. After one-week, *Trichoderma* isolates were applied at 2 g per kg soil. Pots inoculated with *S. rolfsii* but without *Trichoderma* were used as control. The pots were arranged in a completely randomized design with five replications and placed in a screenhouse where mean temperature and relative humidity were around 29 °C and 50 %, respectively. Pots were watered on alternate days with sterilized water. Sclerotia were sampled from soil after one and four months. Twenty-five sclerotia were sampled from each replication. The sclerotia samples were surface sterilized by dipping in 1 % sodium hypochlorite for 1 min, rinsed with sterile distilled water. The viability of sclerotia was evaluated by placing them onto water agar plates for one week at 25 °C and examined for their germination and hyphal growth under a stereomicroscope. Sclerotia was considered viable when hyphae grew out of them.

Statistical analysis

Both experiments were carried out in CRD. Data were statistically analyzed using R studio and mean comparisons were made using DMRT.

RESULTS AND DISCUSSION

Dual culture of *Trichoderma* isolates with *Sclerotium rolfsii*

All the 104 *Trichoderma* isolates and the pathogen *S. rolfsii* grew well on PDA plates within 24 hours. *Trichoderma* isolates grew faster than *S. rolfsii*. Growth of selected 20 *Trichoderma* isolates are presented in Table 2. Area coverage by *Trichoderma* isolates differed significantly. *Trichoderma* isolate T_7, Ramechhap grew faster than the other isolates and covered 17.67 cm^2 within 24 hours.

Table 1. Area covered by 20 selected *Trichoderma* isolates (of total 104 isolates) and *S. rolfsii* in dual culture after 24 h and 48 h of incubation on PDA plates

Trichoderma isolate	Area covered at 24 h*		*Trichoderma* isolate	Area covered at 48 h*		Formation of brown band at contact points
	TI (cm^2)	SR (cm^2)		TI (cm^2)	SR (cm^2)	
T_7, Ramechhap	17.67	7.05	T_{74}, Forest soil	45.62	13.66	-
T_{20}, Bara	15.74	5.96	T_{20}, Bara	44.46	22.91	-
T_{13}, Manang	15.09	4.18	T_{11}, Sarlahi	43.78	14.44	+
T_{11}, Sarlahi	15.09	4.56	T_7, Ramechhap	43.24	20.63	+
T_{62}, Darchula	14.73	9.52	T_{10}, Kavre	42.75	23.69	-
T_{73}, Forest soil	14.55	5.09	T_{72}, Forest soil	41.07	18.12	-
T_{102}, Forest soil	14.51	10.19	T_{13}, Manang	41.05	12.10	+
T_{47}, Makwanpur	14.11	9.58	T_{24}, Dhading	40.91	22.11	+
T_{81}, Forest soil	14.08	8.75	T_{77}, Forest soil	40.51	19.43	-
T_{89}, Forest soil	13.65	7.70	T_{53}, Makwanpur	39.48	21.99	-
T_{100}, Forest soil	13.60	9.73	T_{23}, Dhading	38.27	19.17	-
T_{91}, Forest soil	13.51	7.09	T_{85}, Forest soil	38.16	19.36	-
T_{52}, Makwanpur	13.50	7.11	T_{95}, Forest soil	37.89	20.40	+
T_{77}, Forest soil	13.42	6.95	T_{25}, Manang	36.82	21.06	-
T_{72}, Forest soil	13.36	4.79	T_{48}, Bhaktapur	36.55	23.80	-
$T_{82,}$ Forest soil	13.25	7.76	$T_{26,}$ Jumla	35.91	19.00	-
T_{14}, Sarlahi	12.78	7.45	T_{43}, Chitwan	35.72	17.94	-
T_{29}, Kaski	12.77	7.73	T_{31}, Banke	35.59	20.63	+
T_{63}, Darchula	12.52	6.32	T_{76}, Forest soil	35.44	25.05	-
T_{98}, Forest soil	12.37	7.40	T_{14}, Sarlahi	35.39	23.44	+
SEm±	0.69	0.69		1.38	1.64	
F-test	***	***		***	***	
LSD (≤0.05)	1.94	1.12		3.86	4.587	
CV (%)	12.13	17.85		8.28	15.63	

TI: *Trichoderma* isolate, SR: *S. rolfsii*, SEm: Standard error of the mean, LSD: Least significant difference, CV: Coefficient of variations, Brown band, + : formed; -: not formed.

*The isolates of *Trichoderma* selected for 48 h may be different from the ones for 24 h as the selection was based on their effectiveness for each period of incubation.

After 36 hours of incubation, the *Trichoderma* and *S. rolfsii* came in contact. A brown band was formed after contact of the two colonies in some *Trichoderma* (T_{11}, Sarlahi; T_7, Ramechhap; T_{13}, Manang; T_{24}, Dhading; T_{95}, Forest soil; T_{31}, Banke; and T_{14}, Sarlahi) plates (Table 1), and it was visible from under side of the plate. At 48 hours of incubation, *Trichoderma* isolate T_{74}, Forest soil 15.2 (45.62 cm^2) covered the highest area among all the isolates. There was a significant difference for area coverage among the *Trichoderma* isolates. It was observed that when the area covered by *Trichoderma* increased, the area under *S. rolfsii* decreased (r = -0.9808).

Percentage inhibition of radial growth of *S. rolfsii* by *Trichoderma* isolates

On the fourth day, *S. rolfsii* fully covered the Petri dish in control plates (without *Trichoderma*). *Trichoderma* isolates significantly varied in their ability to inhibit the growth of *S. rolfsii*. The distribution of *Trichoderma* isolates based on their inhibition ability is shown in Figure 1.

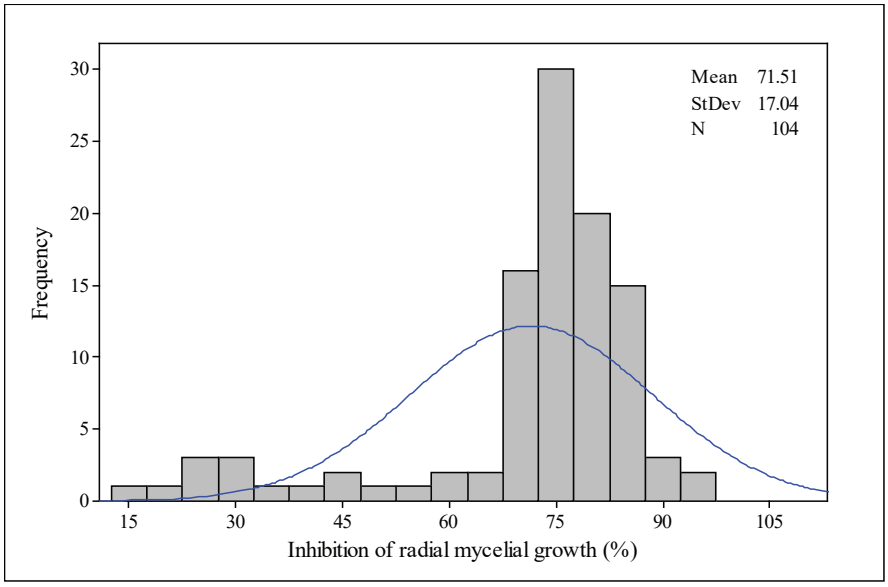


Figure 1. Percentage inhibition of radial growth of *S. rolfsii* by 104 *Trichoderma* isolates

The mean inhibition percentage was 71.24 and varied significantly ($p \leq 0.001$). The majority of isolates (84.31%, n=86) had more than 65% inhibition capacity whereas only 29.41% isolates (n=30) had more than 80% inhibition. Based on the categories, the maximum number of isolates (n=26, 25.49%) had the inhibition percentage of 75-80. Only two isolates had the inhibition percentage of more than 95.

Percentage inhibition of radial mycelial growth of *S. rolfsii* by 20 *Trichoderma* isolates are presented in Table 2. *Trichoderma* isolate $T_{73,}$ Forest soil was the most effective for inhibition of mycelial growth (96.96%) followed by *Trichoderma* isolate $T_{74,}$ Forest soil (94.49%).

Bio-control index of *Trichoderma* isolates on *S. rolfsii*

After eight days of incubation, lysis of *S. rolfsii* mycelium by the majority of *Trichoderma* was observed in dual culture plates. Within 10 days of incubation, only two *Trichoderma* isolates, T_{49}, Bhaktapur and T_9, Kaski completely colonized *S. rolfsii.* While, *S. rolfsii* grew over and completely covered some *Trichoderma* isolates. The distribution of *Trichoderma* isolates based on their biocontrol index is shown in Figure 2.

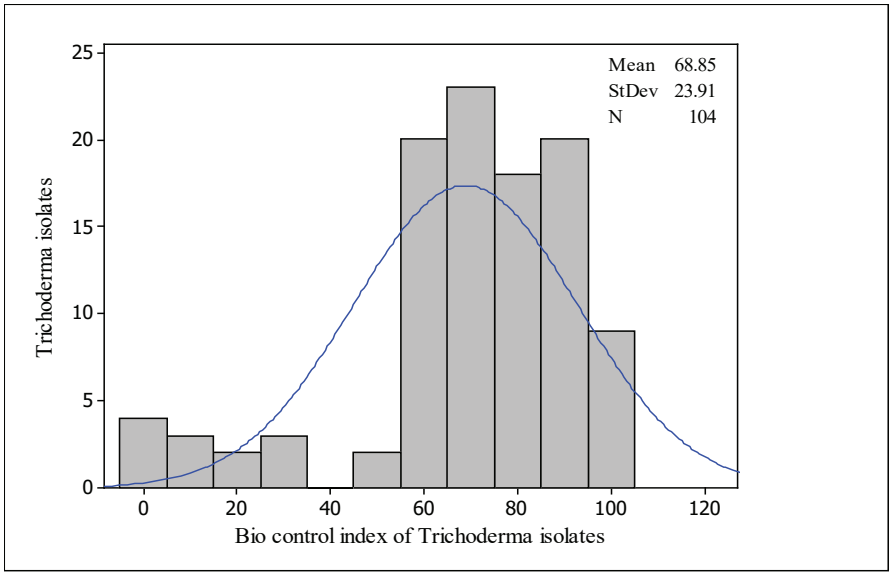


Figure 2. Bio-control index of 104 *Trichoderma* isolates

The mean bio-control index (BCI) was 68.85 and varied significantly ($p \leq 0.001$). The majority of isolates (67.30%, n=70) had bio-control index more than 65 whereas only 27.88% isolates (n=29) had more than 85 BCI. Based on the categories, the maximum number of isolates (n = 33, 31.73 %) had the BCI between 65 and 80. Only eighteen isolates had BCI more than 90.

Also, *Trichoderma* isolates showed differential effects on percent growth inhibition and biocontrol index. The isolate T_{73}, Forest soil showed the highest inhibition (96.96%), while T_{49}, Bhaktapur showed the highest BCI (99) and completely covered colony of *S. rolfsii* (Table 2).

Table 2. Percentage inhibition and bio-control index (BCI) of *Sclerotium rolfsii* by selected 20 *Trichoderma* isolates in dual culture

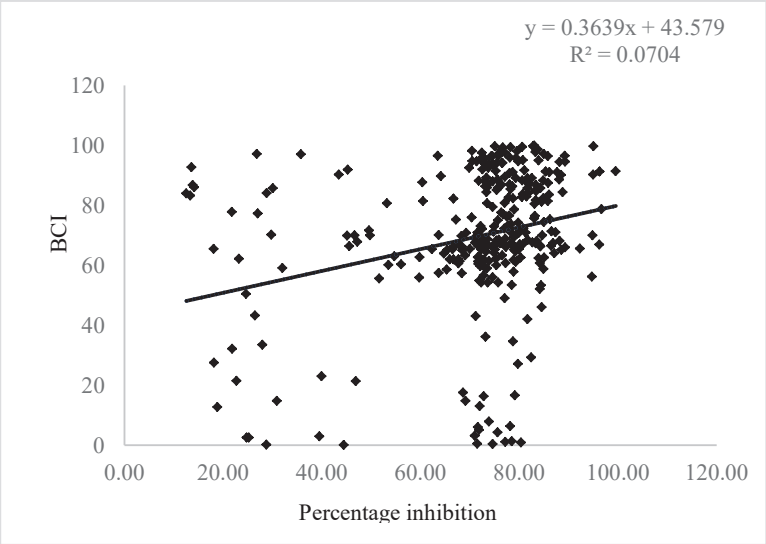
***Trichoderma* isolate**	**PI on 4th day**	***Trichoderma* isolate**	**BCI on 10th day**	**Area of lytic zone (cm^2)**
$T_{73,}$ Forest soil	96.96	T_{49}, Bhaktapur	99.00[a]	17.37
T_{74}, Forest soil	94.49	T_{61}, Gorkha	98.66[ab]	8.89
T_{62}, Darchula	88.92	T_{87}, Forest soil	98.33[ab]	16.56
T_{63}, Darchula	87.93	$T_{70,}$ Forest soil	97.00[abc]	17.79
T_{95}, Forest soil	87.60	T_{48}, Bhaktapur	96.66[a-d]	18.24
T_{72}, Forest soil	87.18	T_{77}, Forest soil	96.33[a-d]	11.43
T_{30}, Palpa	86.16	T_{5}, Tanahun	96.33[a-d]	12.14
T_{53}, Makwanpur	85.11	T_{29}, Kaski	95.66[a-d]	16.42
T_{81}, Forest soil	84.85	T_{84}, Forest soil	95.33[a-d]	14.39
T_{98}, Forest soil	84.82	T_{23}, Dhading	94.33[a-e]	-
T_{59}, Syangja	84.66	T_{59}, Syangja	93.66[a-e]	10.04
T_{85}, Forest soil	84.38	T_{22}, Lalitpur	93.66[a-e]	-
T_{58}, Syangja	84.27	T_{40}, Jumla	93.00[a-f]	18.20
T_{101}, Forest soil	84.26	T_{31}, Banke	92.00[a-g]	8.73
T_{82}, Forest soil	83.89	T_{6}, Parbat	92.00[a-g]	17.86
T_{11}, Sarlahi	83.42	T_{9}, Kaski	90.66[a-h]	19.40
T_{36}, Dang	82.87	$T_{41,}$ Dang	90.66[a-h]	11.49
T_{99}, Forest soil	82.72	T_{62}, Darchula	90.00[a-h]	6.35
T_{88}, Forest soil	82.38	T_{57}, Dolakha	89.33[a-i]	13.30
T_{77}, Forest soil	82.23	T_{67}, Parsa	88.00[a-j]	15.59
SEm±			0.04	
F- test			***	
CV (%)			11.75	

Figures followed by the same letter in column are not significantly different by DMRT. SEm:Standard error of the mean, CV: Coefficient of variations, PI: Percentage inhibition

*The isolates of *Trichoderma* selected for 10th day may be different from the ones for 4th day as the selection was based on their effectiveness for each period of incubation.

Relationship between percentage inhibition and bio-control index

Percent growth inhibition was found positively correlated ($r=0.921$, $p<0.001$) with biocontrol index (Figure 3). It indicated that with a percentage increase in growth inhibition, there was 0.363 percentage increment in BCI.



Figures 3. Relationship between percentage growth inhibition of *S. rolfsii* by *Trichoderma* isolates and biocontrol index (BCI)

In vitro* evaluation of selected *Trichoderma* isolates for sclerotial parasitization of *Sclerotium rolfsii

The effect of selected 30 *Trichoderma* isolates on the germination and parasitization of *S. rolfsii* sclerotia was determined *in vitro*.

On day 2 of incubation, *Trichoderma* isolate T_{31}, Banke parasitized 14 sclerotia out of 25 per plate. On day 3, all *Trichoderma* isolates parasitized more than 20 sclerotia per plate. Some isolates parasitized all 25 sclerotia in the plate. On day 4, all *Trichoderma* isolates parasitized all 25 sclerotia of *S. rolfsii*. After seven days of incubation, most of the sclerotia parasitized by *Trichoderma* isolates became soft and pulpy and when pressed between two fingers, crushed easily (Figure 4a and 4c). However, sclerotia from control plates were found readily germinating when placed on water agar plates.

Evaluation of selected *Trichoderma* isolates for sclerotial parasitization in pot

Based on the *in vitro* results, sclerotial parasitization activity of four potential *Trichoderma* isolates T_{49}, Bhaktapur; T_{31}, Banke; T_{40}, Jumla; and T_{87}, Forest soil were studied in pots under screenhouse conditions. In the first month, sclerotia were formed in the diseased pots (Figure 4b). The sclerotia when plated onto the PDA plates grew well and *Trichoderma* did not grow out of them. After four months sclerotia were again sampled from each pot and plated onto agar medium. In the first two days, some sclerotia germinated and On the third day *Trichoderma* just emerged and started parasitizing the hyphae of *S. rolfsii* (Figure 5). On the fourth day of incubation, lysis of *S. rolfsii* mycelium was clearly seen. On the seventh day, almost all sclerotia collapsed but very few of them were intact.

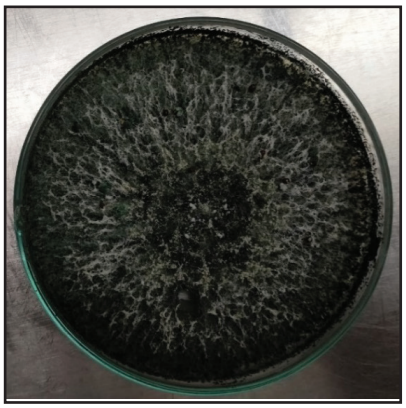


Figure 4a. *Trichoderma* isolate covering sclerotia of *S. rolfsii in vitro* after two days of incubation

Figure 4b. Formation of sclerotia in pots inoculated with *S. rolfsii* and *Trichoderma* isolate

Figure 4c. *Trichoderma* isolate T_{55}, Baitadi and T_{16}, Kavre parasitizng sclerotia of *S. rolfsii in vitro* after three and seven days of incubation, respectively

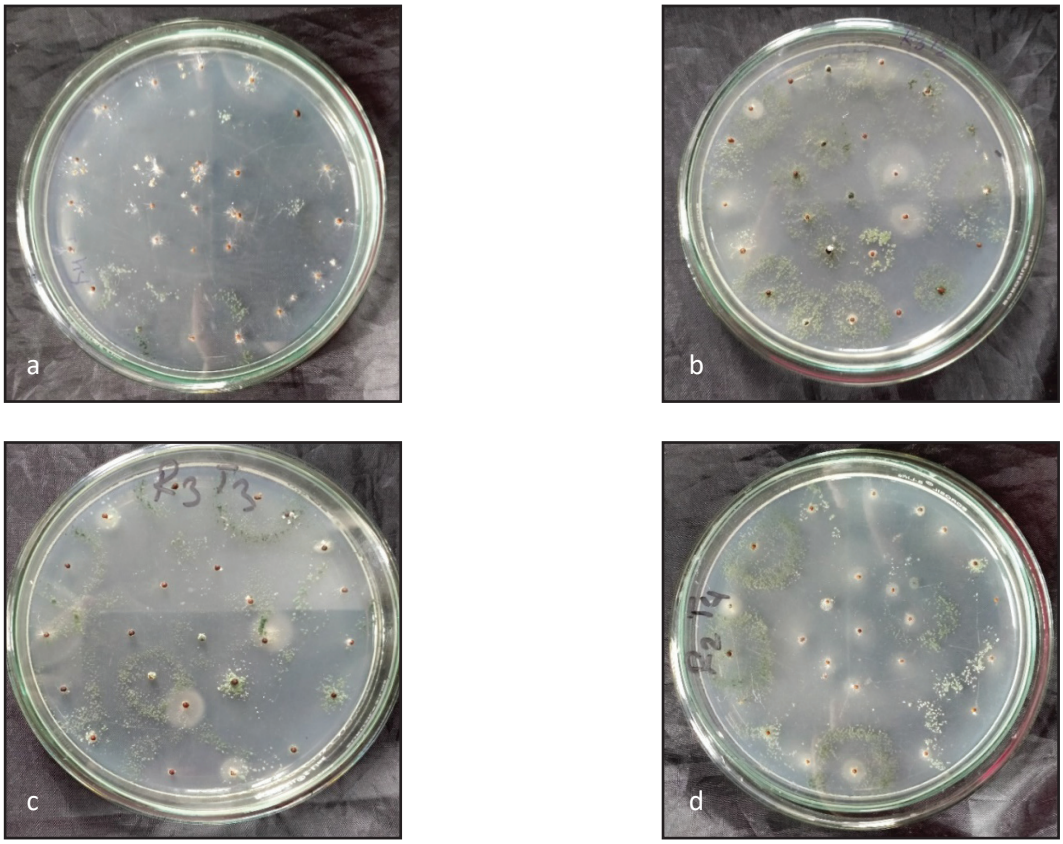


Figure 5. Sclerotial parasitization of *S. rolfsii* by *Trichoderma* isolates: a. T_{87}, Forest soil; b. T_{31}, Banke; c. T_{40}, Jumla; and d. T_{49}, Bhaktapur

The effect of *Trichoderma* on the number of sclerotia germination was not significantly different among the isolates but significantly different from control (Table 3). The number of ungerminated sclerotia was highest (16) in isolate T_{87}, Forest soil and the number of sclerotia with sporulation of *Trichoderma* was highest (22.80) in isolate T_{31}, Banke.

Table 3. Efficiency of *Trichoderma* isolates for sclerotial parasitization

***Trichoderma* isolate**	**Number of ungerminated sclerotia (out of 25)**	**Number of sclerotia parasitized by *Trichoderma***
T_{31}, Banke	14.80[a]	22.80[a] (4.81)
T_{87}, Forest soil	16.00[a]	21.80[a] (4.72)
T_{40}, Jumla	13.20[a]	19.80[a] (4.47)
T_{49}, Bhaktapur	14.60[a]	17.20[a] (4.18)
Control	4.60[b]	0.00[b] (0.70)
SEm±	2.11	0.20
F- test	6.35	0.62
LSD (≤0.05)	*	***
CV (%)	37.50	12.30

Figures followed by the same letter in column are not significantly different by DMRT. Figures in the parentheses are square root transformation values. SEm:Standard error of the mean, LSD: Least significant difference, C.V: Coefficient of variations

Diseases are one of the most important biotic constraints for the successful production of legumes in Nepal. Vascular wilt, collar rot, rust, and Botrytis grey mold are the most serious diseases in lentil. These diseases occur in Terai as well as in the hill areas causing considerable yield losses. Collar rot caused by *Sclerotium rolfsii* is among the economically important diseases of lentil in Nepal. The disease is serious under the rice-based cropping system (Pandey, Yadav, Sah, Pande, & Joshi, 2000). Due to the availability of residual moisture from preceding crop, disease incidence in lentil is high during seedling stage (Roy et al., 2021).

A total of 104 *Trichoderma* isolates were tested *in vitro* for interactions with *S. rolfsii*. Thirty isolates showed an inhibition percentage above 80 %. Some *Trichoderma* isolates formed lytic zones in dual culture plates. The lytic zones were formed due to death of mycelium of *S. rolfsii*. In direct confrontation with fungal pathogens *Trichoderma* employs mycoparasitism and antibiosis (Vinale et al., 2008). In the process of mycoparasitism, *Trichoderma* produces various kinds of cell wall degrading enzymes such as chitinase, cellulose, glucanase etc. Upon secretion of these enzymes by *Trichoderma*, cell walls of the pathogen rupture and oligomers are released (Kubicek et al., 2001; Howell, 2003; Woo et al., 2006; Vinale et al., 2008). Also, competition for carbon, nitrogen and other growth factors along with competition for space plays a significant role in suppression of the pathogen (Howell, 2003; Vinale et al., 2008; and Timila et al., 2015). Similarly, *Trichoderma* produces an abundance of secondary metabolites for survival functions such as metal transport, differentiation, and competition against other microorganisms (Demain & Fang, 2000). These secondary metabolites are natural products and are able to inhibit microbial growth (Vinale et al., 2008).

Not all *Trichoderma* exhibits the similar ability to control the pathogen growth. In this study, maximum number of isolates had the inhibition percentage of 75-80 and only two isolates had the inhibition percentage of more than 95. Khattabi et al. (2004) reported similar results, where they observed that inhibition of *S. rolfsii* by the majority of *Trichoderma* isolates varied between 45 and 55 %. Both the weak and strong inhibitors represented only a small proportion of the total isolates. John et al. (2015) also observed the differential inhibitory action among 10 isolates of *Trichoderma* against *S. rolfsii*. According to Anees et al. (2010) and Scherm et al. (2009) different strains of the same species can exhibit varying levels of inhibition of pathogens and hence, antagonism is not a property of a species. The strains which can express rapidly and efficiently, their genes are involved in antagonistic activities in the presence of the host are in fact better antagonists (Scherm et al., 2009).

An initial rapid increase in area under *Trichoderma* colonies was observed in the first three days, while the area did not change considerably for 4-8 days. After day eight, there was a slight increase in area under *Trichoderma* colonies. Within 10 days of incubation, only two *Trichoderma* isolates, T_{49}, Bhaktapur and T_{9}, Kaski completely parasitized *S. rolfsii*. While, *S. rolfsii* grew over and completely covered some *Trichoderma* isolates. Using the biological control index, the *Trichoderma* strains can be distinguished

according to their antagonistic features against certain plant pathogenic fungi, and it is possible to scale their bio-control abilities. According to Szekeres et al. (2006) when *Trichoderma* are grown along with plant pathogenic fungi for incubation time of 10 days, their mechanisms of antagonism such as competition for space and nutrients, production of antifungal metabolites and mycoparasitism are considered.

In vitro all 30 *Trichoderma* isolates were able to parasitize all 25 sclerotia per plate. Of the 30 isolates, four isolates T_{49}, Bhaktapur; T_{31}, Banke; T_{40}, Jumla; and T_{87}, Forest soil were found promising and studied for their sclerotial parasitization ability in pots under screenhouse conditions. The number of ungerminated sclerotia was highest in T_{87}, Forest soil isolate and number of sclerotia with sporulation of *Trichoderma* was highest in T_{31}, Banke isolate. The results of the present study was in agreement with Henis et al. (1984) as they found germination of sclerotia reduced from 50 to 96 % when sclerotia were treated with *Trichoderma*. Similarly, Khattabi et al. (2004) observed 10.2 to 70.8% of ungerminated sclerotia in natural soil and 50.5 to 92.6% in sterilized soil when treated with *Trichoderma*. In the present study, we found 100 % germination of sclerotia in soil (pot culture) when sampled after 30 days. And the germination reduced upto 88 % in four months. The result was in agreement with Santos & Dhingra (1982) who observed that the number of viable sclerotia of *Sclerotinia sclerotiorum* when treated with *Trichoderma* decreased with increasing incubation period. They also reported that the decrease in viable sclerotia with time was more rapid by *T. koningii* than by *T. harzianum*. There are reports which showed that the different strains of *Trichoderma* spp. varied in their ability to parasitize the sclerotia of *S. rolfsii* (Henis et al., Papvizas & Lewis, 1989; Bhagat & Pan, 2011; Sarrocco et al., 2006). According to Sarrocco et al. (2006) and Köhl et al. (2019) some *Trichoderma* species are able to penetrate the rind and parasitize the inner cell layers of sclerotia, leading to destruction and leaving them not viable. Rawat & Tewari (2010) reported deformation, lysis and degradation and disappearance of cytoplasmic granules of cell wall of sclerotia. *Trichoderma* can induce enzymatic degradation of rind walls and degrade melanin of the sclerotia (Sarrocco et al., 2006). According to Butler et al. (2005) among different mechanisms of action in biocontrol, destruction of the melanin or inhibition of its synthesis is an important one.

CONCLUSION

In the present study, *Trichoderma* isolates $T_{49,}$ Bhaktapur and T_{87}, Forest soil showed promising results in controlling *S. rolfsii in vitro*. Both isolates were also able to parasitize and reduce the germination of sclerotia present in the soil. It can be stated that these isolates of *Trichoderma* could be used successfully to control diseases caused by *S. rolfsii*. Further studies regarding their efficacy under field conditions are needed.

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